Low iron requirement for growth in oceanic phytoplankton

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DESPITE the controversy on the importance of iron in limiting phytoplankton growth and affecting air–sea exchange of CO_2 in the ocean[1–4], there is very little information on cellular iron requirements for growth. The few data available[5,6] come from species isolated from coastal sea water where dissolved Fe levels are 10–1,000 times higher than those ($\leq$0.1 nM) in the open ocean[1,7]. Species from oceanic waters require much lower external Fe concentrations for growth than do comparable coastal species[8]. Here we report that an oceanic diatom was able to grow at a near maximum specific rate of about 1.0 per day at a cellular Fe:C ratio of 2 μmol:mol, about 25% of the amount needed for the same rate in a related estuarine species, and 2–20% of values previously used to estimate algal Fe requirements in sea water[1,2]. These results have important implications concerning iron limitation of primary productivity in the ocean and cell biology of iron in oceanic algae.

We measured the effect of Fe concentration on cellular Fe:C ratio and growth rate in *Thalassiosira oceanica* (clone 13-1), isolated from the Sargasso Sea, and *T. pseudonana* (clone 3H)

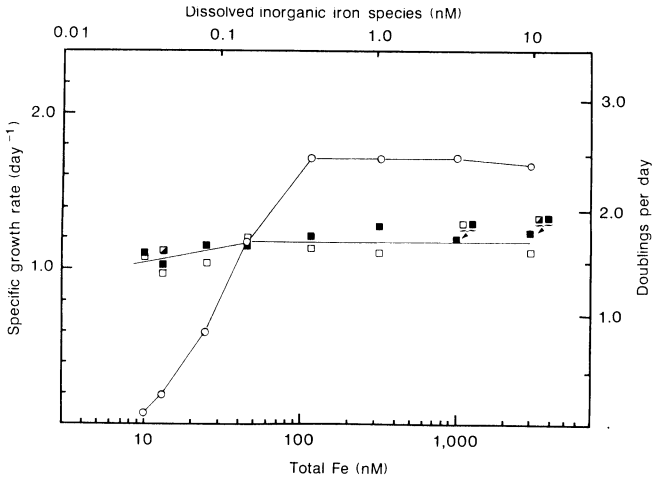

FIG. 1 Growth rate of clones 3H (○) and 13-1 (three successive experiments (◪, ■, □)) as a function of total Fe and mean concentration of dissolved inorganic Fe species. Cells were preacclimated at low total Fe (10 nM for 13-1, 45 nM for 3H) for 7–19 days and grown at 20 °C and pH 8.10 ± 0.06 under 14:10 h light:dark cycle (600 μmol quanta m^{-2} s^{-1}) in filtered (0.4 μm) Gulf Stream sea water (stored in the dark at 7 °C for 1 year). Sea water contained added nutrients (35 μM $NaNO_3$, 1.5 μM Na_2HPO_4, 40 μM Na_2SiO_3, 0.1 μg l^{-1} vitamin B_{12}) and a trace metal ion buffer system (0.1 mM EDTA, 100 nM $CuCl_2$, 250 nM $ZnCl_2$, 120 nM $MnCl_2$, 100 nM $CoCl_2$). Total Fe was computed from the sum of the background concentration (10 nM as measured by atomic absorption spectrophotometry following organic extraction[18,19]) plus Fe added with radiotracer and as unlabelled $FeCl_3$. Log free-ion concentrations of Cu^{2+}, Zn^{2+}, Co^{2+} and Mn^{2+}, were computed from equilibrium theory[11,20] and are −13.12, −10.42, −10.63, and −8.14, respectively. The mean dissolved inorganic Fe concentration [Fe′] was computed from conditional formation, dissociation and photodissociation rate constants for iron-EDTA in sea water[5]. Computed [Fe′]/[total Fe] = 0.00077 in dark and 0.0053 in light giving weighted mean of 0.0034 for 14:10-h light:dark cycle.

from a eutrophic estuary[9]. Experimental procedures and conditions were similar to those in previous culture studies with ^{54}Mn (refs 10, 11). Algae were grown in filtered Gulf Stream sea water containing added nutrients, $FeCl_3$ radiolabelled with ^{55}Fe and an EDTA-trace metal ion buffer system that reduced available inorganic Fe to levels far below total Fe concentrations. Growth rate was measured from daily increases in total cell volume using a Coulter counter. Intracellular Fe was determined in exponentially growing cells by filtering them onto 3-μm-pore Nucleopore filters, rinsing briefly with a Ti(III)EDTA-citrate reducing solution[12] to dissolve iron hydroxides and desorb ferric ions bound to cell surfaces, and measuring the remaining particulate ^{55}Fe by liquid scintillation counting. In some instances the cells were rinsed with sea water instead of reducing solution to measure total (intracellular plus surface) cell Fe. Cell ^{55}Fe values were corrected with filter blanks using media without cells. The fraction of ^{55}Fe in the cells was multiplied by the total Fe concentration and divided by the measured volume of cells per litre to yield cell Fe concentrations. These were converted to Fe:C ratios using cell carbon:volume ratios of 22 and 15 mol C l^{-1} for clones 3H and 13-1 that we determined with standard ^{14}C techniques and Coulter counter measurements of cell volume. Carbon:volume ratios were unaffected by Fe concentration (data not shown).

Decreases in Fe concentration reduced growth rate more in the neritic clone 3H than in the oceanic clone 13-1 (Fig. 1) in accordance with previous results[8]. Clone 3H grew 50% faster than clone 13-1 at high Fe concentrations; but at the lowest Fe level, its growth rate decreased to near zero while that of clone 13-1 was reduced by only about 15%. The ability of clone 13-1 to grow faster than clone 3H at low Fe levels was due almost entirely to a much lower cellular iron requirement for growth (Fig. 2), and not to a greater ability to accumulate iron (Fig. 3*a*). When intracellular Fe:C ratios were multiplied by specific growth rates, the resultant specific cellular Fe uptake rates were very similar for the two species at low growth-limiting Fe levels (Fig. 3*b*).

Oceanic algae might have been expected to have evolved higher affinity transport systems to acquire iron more effectively at low concentrations, as observed for other nutrients (for example Mn[11] and nitrate[13]). But Hudson and Morel[5] recently reported that Fe-uptake kinetics in two coastal phytoplankters approach the physical limits for diffusion of inorganic Fe species to the cell surface and for kinetics of Fe-ligand exchange at membrane transport sites. They predicted that oceanic algae could not have higher transport kinetics and that the only available means of adaptation to low-iron oceanic conditions would be a reduction in cell Fe requirement or size. A reduction in size does not apply in this case because clone 13-1 is larger than clone 3H; mean cell volumes were 139 ± 12 (±s.d.) and

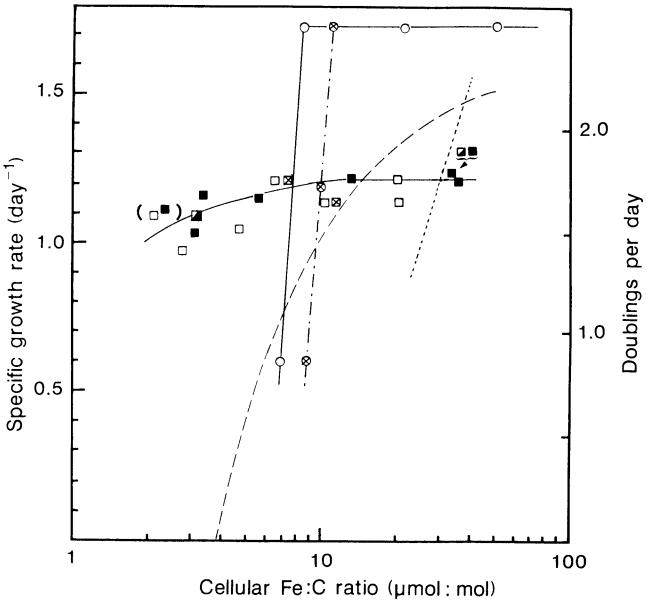

FIG. 2 Specific growth rate versus cell Fe:C ratio for a single 3H experiment based on intracellular (○) and total cell (⊗) Fe, for two 13-1 experiments based on intracellular Fe (◪, ■) and a third based on intra- (□) and total (⊠) cell Fe. Ratios of intra- to total cell Fe were 0.89 ± 0.005 and 0.77 ± 0.1 (±range, $n = 2$) for 13-1 and 3H at Fe concentrations ≤110 nM where $Fe(OH)_3$ does not precipitate. Data points in parentheses (■, □) are estimates for cultures without added Fe or ^{55}Fe, extrapolated from specific growth rate and relationships among cell Fe uptake rate, specific growth rate and Fe concentration (Figs 1 and 3; refs 11, 20). Cells were inoculated at 0.3 μmol cell C l^{-1} and grown for about 7 generations before measurement. Cell Fe levels were measured in narrow biomass range of 29–75 μmol cell C l^{-1} to have sufficient cell mass for accurate measurement without having enough to appreciably affect dissolved Fe chemistry or culture pH (ref. 5). Dashed line is data for *T. weissflogii* from Harrison and Morel[6] measured at 20 °C in continuous light normalized to cell C using our measured C:volume ratio of 5.6 mol l^{-1}. Dotted line is estimate of cell Fe:C growth requirement based on biochemical maximum use efficiency calculations of Raven[14] at 20 °C and saturating light. His specific growth rates computed for continuous light were multiplied by 0.58 to adjust to our 14:10 h light:dark cycle.

$32 \pm 4.7\ \mu m^3$, respectively. Our results confirm their predictions.

The amount of cellular Fe required for near maximum growth of clone 13-1 is much lower than minimum amounts thought necessary to meet the metabolic needs of plant cells[14]. It is $\leq 10\%$ of calculated amounts needed for growth based on Fe enzymatic requirements in photosynthesis, respiration and NO_3 reduction (Fig. 2). It is also 10–100 times less than values previously used to estimate algal growth requirements for Fe in sea water [1,2]. These estimates were based on laboratory experiments with unacclimated cultures of the coastal species *T. weissflogii*[15] and on amounts of particulate Fe in near-surface sea water after subtracting amounts leachable by weak acid and estimated to occur in aluminosilicate minerals[2]. These latter values are uncertain as they do not correct for iron adsorbed to particles or present in iron oxides. On the basis of these values, it has been concluded that ratios of Fe : NO_3 in upwelling sea water were 10–100 times too low to meet the matabolic needs of phytoplankton and, therefore, that most of the Fe required for growth must be supplied from atmospheric deposition[1,2].

This conclusion needs to be reassessed in light of our findings. Dissolved Fe within the nutricline of the North Pacific is highly correlated with NO_3, PO_4 and SiO_3 concentrations[1,2], suggesting that it is regulated by biological uptake and regeneration processes as occurs for major nutrients. For this to be true, however, the relative changes in dissolved Fe and major nutrients with depth should reflect the concentrations of these elements in phytoplankton, the major biomass reservoir. Linear regressions between dissolved Fe and NO_3 in the nutriclines of four North Pacific stations[2] yield slopes of 13.5, 15.0, 11.3 and 13.8 μmol Fe : mol NO_3 (coefficient of variation, $r^2 = 0.98, 0.94, 0.96, 0.90$) which translate to a mean Fe : C ratio of 2.0 ± 0.2 μmol : mol, assuming a typical 6.6 : 1 C : N ratio in plankton[16]. This Fe : C ratio would support a specific growth rate of clone 13-1 of about 1.0 per day, suggesting that there could be enough dissolved Fe in deep water when it is advected to the surface to support low to moderate growth rates of oceanic species, assuming most of the Fe is biologically available.

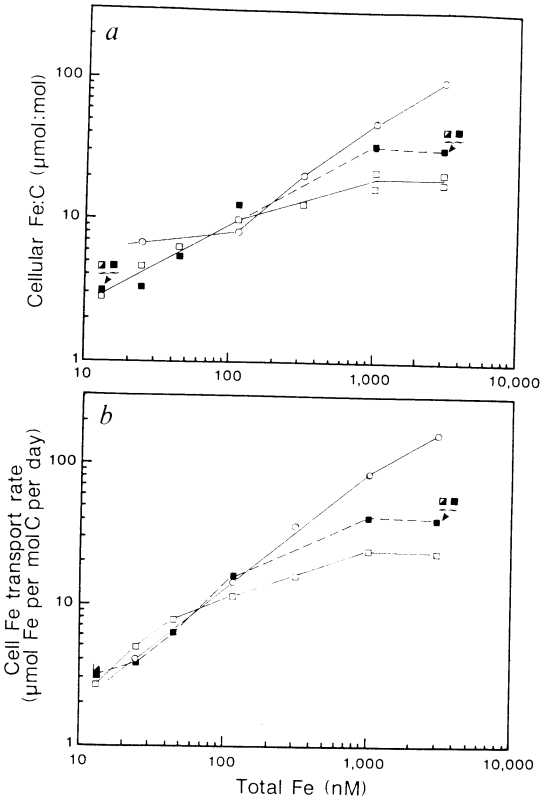

FIG. 3 *a*, Intracellular Fe:C ratios in clones 3H and 13-1 as functions of total Fe in medium. *b*, Specific Fe transport rates were computed by multiplying intracellular Fe:C ratios by specific growth rates. ○, 3H; ◪, ■ and □, three experiments with 13-1.

Although the Fe : C ratio derived from dissolved Fe to major nutrient correlations will support growth of the oceanic species, it is too low for growth of coastal diatoms *T. pseudonana* and *T. weissflogii* (Fig. 2). Thus, for a hypothetical algal community composed of these three species, the coastal diatoms would be selected against under oceanic low-Fe conditions and the community would be dominated by the slower growing oceanic diatom. Increasing the Fe concentration might have little immediate effect on community growth rate as the oceanic species might be growing near its maximum; but after a period the rate would increase as the community became dominated by the high-Fe species with faster maximum rates. This would explain observations from algal growth experiments in surface water from the subarctic Pacific[2,4] and Southern Ocean[3] in which Fe additions caused little or no effect for the first 2–4 days, but stimulated growth and caused associated shifts in species dominance after this period.

Data from the experiments in the subarctic Pacific[2] support our findings of the low cellular Fe : C growth requirements of oceanic algae. There was some growth in all of the controls (with no Fe added) in these experiments, despite low Fe concentrations in the water. At station T-6, for example, suspended particulate matter increased by 0.814 mg kg^{-1} (6.2-fold), which apparently was due to phytoplankton growth as it was accompanied by a 7.4-fold increase in chlorophyll. Dissolved Fe was 0.08 nM, and if all of it were taken up by the growing phytoplankton, we compute a maximum cellular Fe : C ratio of 2.6 μmol : mol (based on a 0.45 cellular C : dry weight ratio[14]). We compute a similar value (2.9 μmol : mol) if we base our growth estimates on the amount of NO_3 taken up by the cells (4.2 μM) and a C : N ratio for plankton of 6.6 (ref. 16). These estimates agree well with our growth requirements for clone 13-1 and with the Fe : C ratio (2.3 μmol : mol) derived from the relative increases in dissolved Fe and NO_3 within the nutriciline at this station as discussed above.

The results of our investigation, the covarying distributions of dissolved Fe and major nutrients, and the results of shipboard growth experiments all indicate that Fe is an important biologically controlling nutrient in the sea whose distribution is influenced by phytoplankton uptake and regeneration processes. Whether it is more important in controlling algal community growth rate and species composition than traditional major nutrients is unknown, and is currently being actively debated[17]. □

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